Augmented force output in skeletal muscle fibres of *Xenopus* following a preceding bout of activity

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1. The effect of a brief period of activity on subsequent isometric tetanic force production was investigated in single muscle fibres of *Xenopus laevis*.

2. Following a train of ten tetani separated by 4 s intervals, tetanic force was significantly augmented by about 10%. The tetanic force augmentation persisted for at least 15 min and then slowly subsided. A similar potentiation was seen following trains of five and twenty tetani.

3. During the period of tetanic force potentiation, tetanic calcium was reduced by more than 30%, and intracellular pH was reduced from 7·15 ± 0·07 to 7·03 ± 0·11 ($n = 4$).

4. Fibre swelling was greatest at 1 min and then subsided over 15–20 min and possibly accounted for a small part of the observed force potentiation.

5. A reduction in the inorganic phosphate (P_i) concentration of more than 40% was found in fibres frozen in liquid nitrogen at the peak of force potentiation compared with resting fibres.

6. It is concluded that the augmentation of tetanic force found after a brief preceding bout of activity is due to a reduction in inorganic phosphate. This mechanism may underlie the improved performance observed in athletes after warm-up.

A period of physical activity, in various forms and of variable duration, before vigorous exercise (popularly known as warming-up and commonly used by athletes) improves performance (McArdle, Katch & Katch, 1991). Numerous explanations of this effect have been advanced including increased body and muscle temperature (Beelen & Sargeant, 1991), stimulation of the cardio-respiratory system (Barnard, Gardner, Diaco, MacAlpin & Kattus, 1973) and increased elasticity of tendons and connective tissue (Safran, Garrett, Seaber, Glisson & Ribbeck, 1988).

Regardless of the systemic changes, which occur as a result of warming-up, there is also the likelihood that changes within the muscle fibres themselves contribute to the improved muscle performance. A likely candidate is a change in inorganic phosphate (P_i) concentration. It is well established that elevation of P_i depresses force production in skinned muscle fibres and the relationship between P_i and force is curvilinear, such that for a given increase in P_i, force is depressed more when the initial P_i concentration is low (e.g. Pate & Cooke, 1989; Millar & Homsher, 1990; Stienen, Roosemalen, Wilson & Elzinga, 1990). Thus, in intact fibres, lowering P_i to less than its usual resting value might cause a substantial augmentation of the force production. Recently it was reported that following brief bouts of exercise (similar to those used in a warm-up regimen), P_i was reduced below pre-exercise values (Yoshida & Watari, 1993*a*, *b*). However, these studies concentrated on the metabolic consequences of short periods of moderate exercise and did not report on changes in contractile properties of the muscles.

In this study, we show that a brief bout of preceding activity augments subsequent force production in isolated muscle fibres. This increased force is due mainly to a reduction in P_i in the muscle fibres and can be the explanation for the improved performance with warm-up.

METHODS

General

Xenopus laevis frogs were stunned, decapitated and pithed. Single muscle fibres were then isolated from the lumbrical muscles. Fibres were mounted in a chamber (volume about 1 ml) and their length was adjusted so that isometric tetanic force was maximal. Supramaximal stimuli (70 Hz, 400 ms) were applied via platinum plate electrodes and the resultant tetanic force responses were detected by an Akers AE 801 force transducer, amplified and recorded on a pen recorder and personal computer. In some experiments the maximum shortening velocity (V_0) was measured. One tendon was then attached to the arm of a galvanometer which could be rapidly moved by changing the current through the

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galvanometer coil; a 200 μm step was completed within 0·9 ms. After ensuring that tetanic force was stable, a series of tetani, usually ten, with 4 s intervals between successive tetani was applied to the fibre.

Values are presented as means ± S.E.M. Paired t tests were performed to verify statistical significance; the significance level was set to 0·05 except when stated otherwise.

Solutions

Fibres were superfused (8 ml min^{-1}) with a Ringer solution containing (mM): NaCl, 115; KCl, 2·5; $CaCl_2$, 1·8; sodium phosphate, 3·0 (pH, 7·0). Temperature in the chamber was monitored 1 mm from one end of the fibre and was maintained constant at 20 ± 0·1 °C by means of a feedback-controlled heat exchange device.

Measurement of fibre swelling

In some experiments the cross-sectional area was measured; since the fibre length was kept constant, changes in the cross-sectional area corresponded to changes in fibre volume. For this purpose, a short segment (about 20 μm) of a vertically mounted fibre was illuminated from the side with white light emerging from a slit and focused on the fibre (Blinks, 1965; Lännergren, 1990). The illuminated segment was viewed from above with a long-working distance objective and monitored using a video camera system. Fibres were not stimulated during recovery to ensure that the same cross-section was kept in focus. Changes resulting from twenty tetani were measured instead of the usual ten tetani because it was felt that the changes in cross-sectional area would have otherwise been too small to be measured accurately.

Measurement of $[Ca^{2+}]$ and $[H^+]$

The myoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured with the fluorescent Ca^{2+} indicator indo-1; the methods for measuring fluorescence signals and converting these into $[Ca^{2+}]_i$ are given in Westerblad & Allen (1993, 1994).

The fluorescent pH indicator carboxy-SNARF-1 was used to measure intracellular pH (pH_i). Fibres were incubated for 8–12 min in 10 μM of the membrane-permeant acetoxymethyl ester form of the dye. During experiments fibres were excited with light from a Xenon lamp which passed through a 540 ± 10 nm interference filter. The emitted light was measured at 580 and 640 nm with two photomultiplier tubes and the pH_i-dependent signal was obtained as the ratio of the signal at 640 nm to that at 580 nm. To avoid bleaching of the dye, the intensity of the excitation light was kept as low as possible and a shutter prevented the light from reaching the preparation except during brief periods of measurement (see Fig. 5). To be able to translate fluorescence ratios into pH_i, we performed an intracellular calibration at the end of experiments employing the proton and potassium ionophore nigericin (Buckler & Vaughan-Jones, 1990). Fibres were first exposed to a solution consisting of (mM): KCl, 140; $MgCl_2$, 1; KH_2PO_4, 1·4; Hepes, 10; EGTA, 1. This resulted in a brief contracture and 20 μM nigericin was thereafter applied and pH was varied between 6 and 8·5 in steps of 0·5 pH. Fibres were exposed to each pH until a stable fluorescence ratio was obtained. Ratios obtained at the different pH values showed little variation between experiments.

Measurement of inorganic phosphate

Bundles of two to eight fibres were isolated from *Xenopus laevis* iliofibularis muscles. Fibre bundles were given a series of ten tetani and were frozen in liquid nitrogen 7 min after this series (i.e. after the demonstration of tetanic force potentiation). Two types of control bundles were used: (1) bundles mounted in the experimental chamber and frozen after a 90 min rest period and (2) bundles first given a train of ten stimuli and then frozen after the force potentiation had subsided and an additional 30–60 min rest period. After freeze-drying, tendons were removed and the fibres weighed. Sufficient material was available to do multiple separate determinations of P_i in control fibres. However, in order to have sufficient material to determine P_i in fibres frozen immediately after they had demonstrated potentiation, it was necessary to pool all the available bundles. Pooled muscle samples weighing 0·23–0·41 mg were extracted with 0·5 M perchloric acid, centrifuged and the supernatant neutralized with $KHCO_3$. The samples were again centrifuged and the supernatants analysed for P_i by fluorimetry with an enzymatic technique coupled to a pyridine nucleotide system (Lowry & Passoneau, 1972). The investigator performing the analyses was unaware of the experimental conditions to which the fibres were subjected until after the analyses were completed.

RESULTS

Tetanic force fell by about 10% between the first and last tetani of the standard tetanus series and was still less than 100% 1 min after the end of the series. However, at 5 min a significant potentiation of tetanic force averaging 9·4 ± 1·6% ($n = 10$) was seen (Fig. 1). A significant augmentation persisted for at least 15 min. Tetanic force returned completely to the baseline level in all fibres by 50 min. The potentiation after ten tetani was seen in both frog single toe fibres and small bundles of iliofibularis fibres. In further experiments, a series of five or twenty tetani and also a series of ten 15 Hz sub-tetanic stimuli produced a potentiation of similar size.

Force potentiation cannot be explained by fibre swelling

Tetanic force has been found to be augmented in fibres whose volume is increased by exposure to hypotonic solution (Edman & Hwang, 1977). In addition, fibre volume is reported to increase after repeated tetanic contractions (Gonzalez-Serratos, Somlyo, McClennan, Shuman, Borrero & Somlyo, 1978; Lännergren, 1990). Therefore, we measured the changes in fibre volume resulting from a series of twenty tetani (Fig. 2). Although fibre volume increased after the series of tetani, no temporal relationship was apparent between volume changes and potentiation of force. For example, the increase in fibre volume was maximal 1 min after the end of stimulation and had declined to less than 1% by 15 min, whereas potentiation of force was greatest at 5 min post-stimulation and was still marked at 15 min.

In a further test of the involvement of muscle fibre swelling, fibres were exposed to a Ringer solution containing only 90% of the usual NaCl concentration which increases fibre volume by about 6% (Blinks, 1965). We observed a maximum increase in fibre volume of about 10% with twenty tetani (Fig. 2) and a set of experiments using forty tetani gave increases ~2-fold those obtained with twenty tetani (data not shown). Thus, the increase in fibre volume produced by exposure to 90% NaCl hypotonic

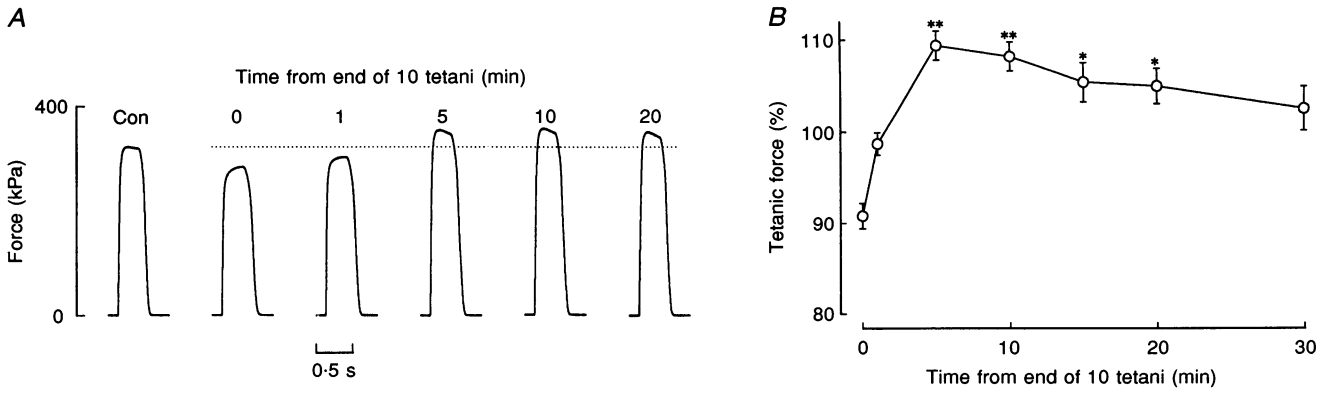

Figure 1. Prior application of 10 tetani causes potentiation of tetanic force after a brief delay

A, typical force records showing that maximum potentiation of tetani occurred at 5 min. Tetanic force declines during the series of 10 tetani, from the first (Con) to the last (0 min) tetani. The dotted line is a reference line which indicates peak force obtained in the first (Con) tetanus. *B*, mean value (± S.E.M.) of 10 fibres showing that potentiation occurs with a delay and that force is significantly greater than control for at least 15 min (* $P < 0{\cdot}05$; ** $P < 0{\cdot}005$).

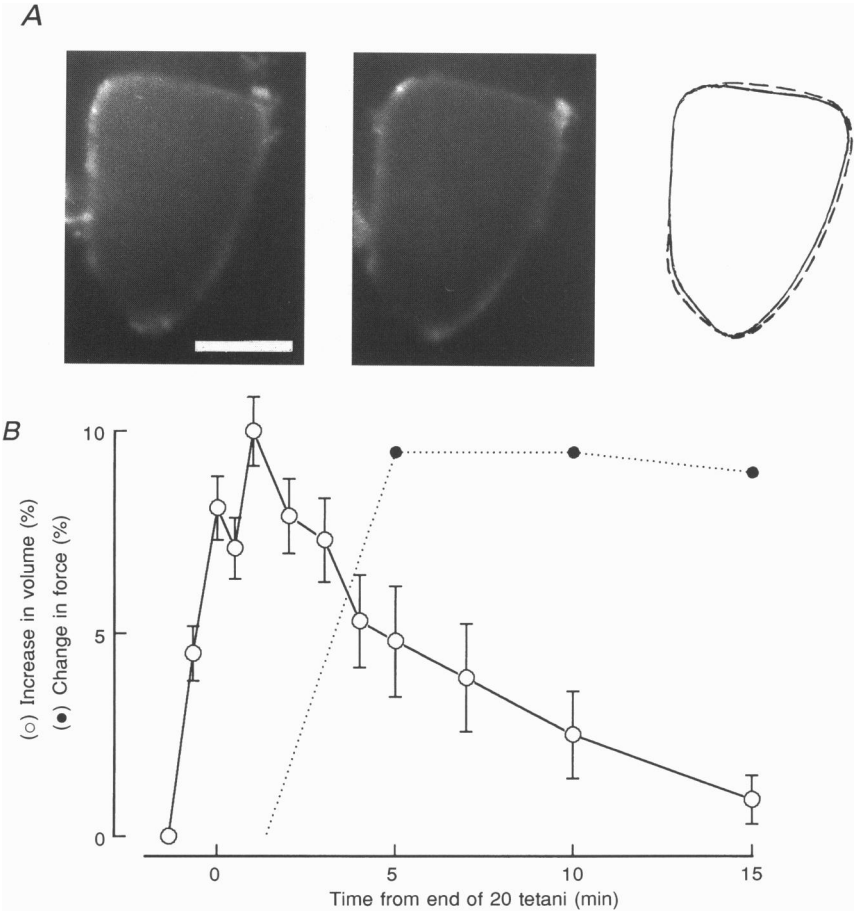

Figure 2. Volume changes cannot explain the observed force potentiation

A, cross-section of a fibre in the rested state (left) and 5 min after 20 tetani at 4 s intervals (middle), and line tracings of the cross-sections (right; full line is control and dashed line is after stimulation). Calibration bar = 50 μm. *B*, time course of volume changes of isolated fibres (○; $n = 6$) calculated from changes in cross-sectional area. ●, the average time course of potentiation in two other fibres also given 20 tetani. Note that the time course of potentiation is markedly different from that of volume changes.

solution is likely to be the maximum swelling which occurs in a fibre after ten tetani, and hence markedly larger than the expected swelling at 5 to 15 min (i.e. the period when maximum force potentiation occurred). As a result of exposure to hypotonic solution, tetanic force increased by $3{\cdot}0 \pm 0{\cdot}6\%$ ($n = 4$). Thus, fibre swelling contributes little to the observed potentiation.

Potentiation is not due to increased tetanic $[Ca^{2+}]_i$

To rule out the possibility that calcium release was not saturating in control tetani, fibres were exposed to caffeine which facilitates calcium release from the sarcoplasmic reticulum (Klein, Simon & Schneider, 1990). Fibres exposed to 0·5 mM caffeine did not show any significant change in tetanic force ($-1{\cdot}0 \pm 0{\cdot}84\%$; $n = 5$).

Figure 3 shows typical records of $[Ca^{2+}]_i$ and force from one fibre before and 5 min after a series of ten tetani. Observe that tetanic $[Ca^{2+}]_i$ was actually reduced at the time of force potentiation. Data from four fibres showed that tetanic $[Ca^{2+}]_i$ was more than 30% lower during potentiation ($1{\cdot}81 \pm 0{\cdot}17$ μM) compared with control ($2{\cdot}63 \pm 0{\cdot}25$ μM). Resting $[Ca^{2+}]_i$ was $47{\cdot}5 \pm 3{\cdot}1$ nM and $63{\cdot}5 \pm 7{\cdot}1$ nM before and 5 min after the series of ten tetani, respectively. These results show clearly that calcium release is not augmented during the period of potentiation.

Possible metabolic causes of force augmentation

Muscle activity alters the concentration of protons and P_i and both these metabolites have large impacts on contractile function. Elevated H^+ decreases both force production and shortening velocity (Metzger & Moss, 1987). Increased P_i, on the other hand, reduces force output but has little effect on shortening velocity (Pate & Cooke, 1989). Thus, lower than usual levels of H^+ and P_i might account for the observed force potentiation and it should be possible to distinguish between these two by measuring the maximum shortening velocity (V_0). Figure 4 shows an example where V_0 was assessed in control and during the period of potentiation. It can be seen that V_0 was almost identical in the two situations and similar results were obtained in the other fibres tested giving a V_0 of $6{\cdot}7 \pm 0{\cdot}4$ fibre lengths s^{-1} in control and $6{\cdot}6 \pm 0{\cdot}4$ fibre lengths s^{-1} ($n = 5$) during potentiation.

Figure 5 shows a typical example of how pH_i changes during and after a series of ten tetani. In resting fibres pH_i was $7{\cdot}15 \pm 0{\cdot}07$ ($n = 7$) and increased to $7{\cdot}29 \pm 0{\cdot}08$ by the fifth tetanus. After the series of ten tetani, pH_i reached a minimum of $6{\cdot}99 \pm 0{\cdot}14$ during the first minute and then recovered slowly. However at 5 min, when force was potentiated, pH_i was $7{\cdot}03 \pm 0{\cdot}11$, still 0·12 pH units lower

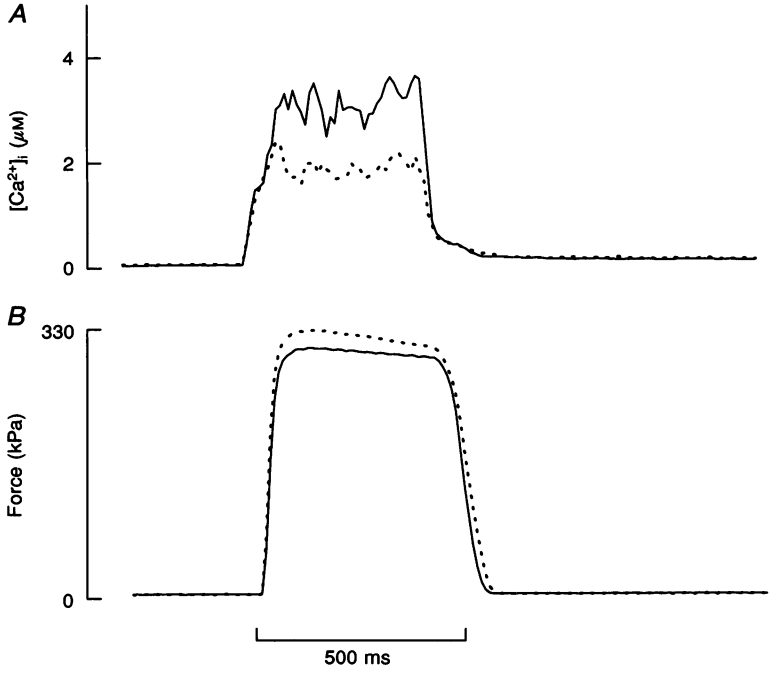

Figure 3. Force potentiation is not due to an increased tetanic $[Ca^{2+}]_i$

A, typical records of $[Ca^{2+}]_i$ obtained in the same muscle fibre during the control period (continuous line) and at 5 min after a series of 10 tetani (dashed line). Note that the calcium transient at 5 min is reduced compared with control. *B*, tetanic force resulting from the calcium transients shown in *A*. Continuous line is the control tetanus while the dashed line represents the augmented tetanus occurring at 5 min after the series of 10 tetani.

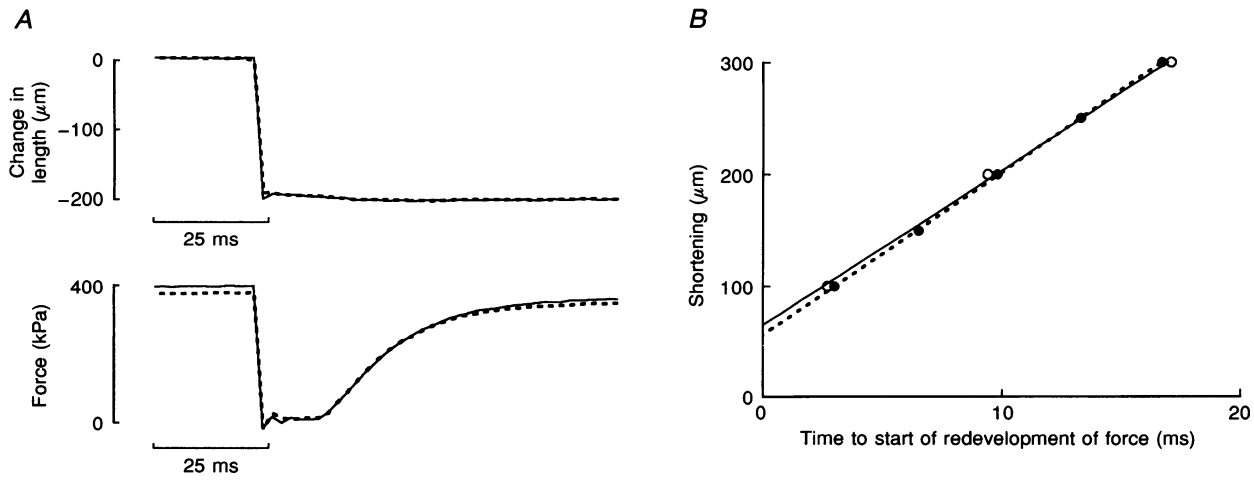

Figure 4. Shortening velocity is not altered in the potentiated state

A, typical experiment illustrating redevelopment of force after a rapid shortening in both the rested (dashed line) and potentiated states (continuous line). Top traces show muscle length with a step change of 200 μm and bottom traces show the force response to this step change. Note that time to redevelopment of force is virtually identical in the rested and potentiated states. *B*, maximum shortening velocity (V_0, slope of the fitted lines) and series elasticity (intercept on the ordinate) during the period of potentiation (●) are not significantly different from those in the rested state (○); same fibre as in *A*.

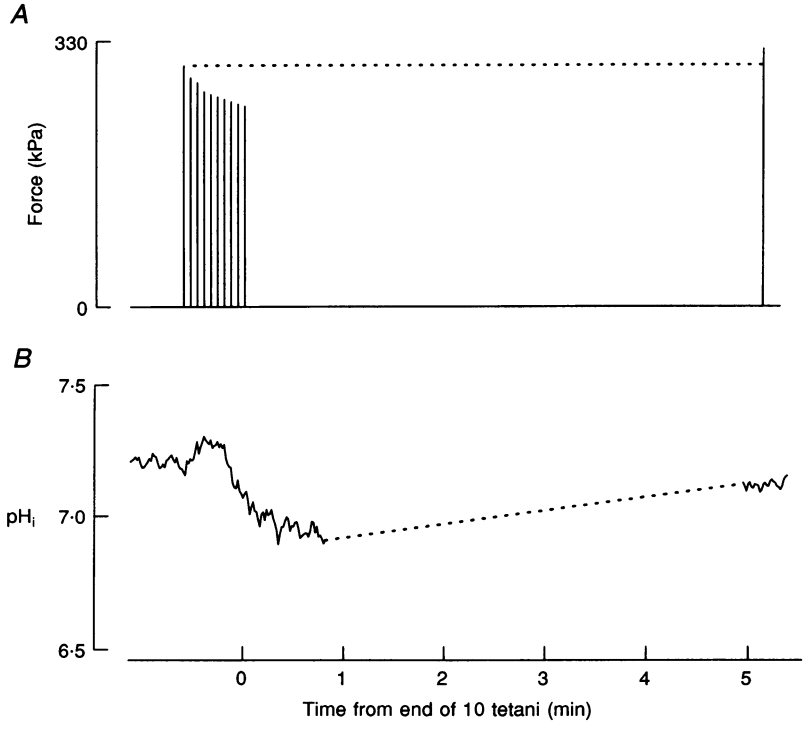

Figure 5. Intracellular pH is lower than control when force is potentiated

A, force record showing the series of 10 tetani and potentiated tetanus at 5 min. Dashed line is a reference indicating force of the control tetanus. *B*, changes in intracellular pH measured with SNARF-1 in the fibre depicted in *A*. Note that pH$_i$ is less than the resting level at the time of force potentiation. Dashed line indicates the period when the light was switched off to avoid bleaching of the dye.

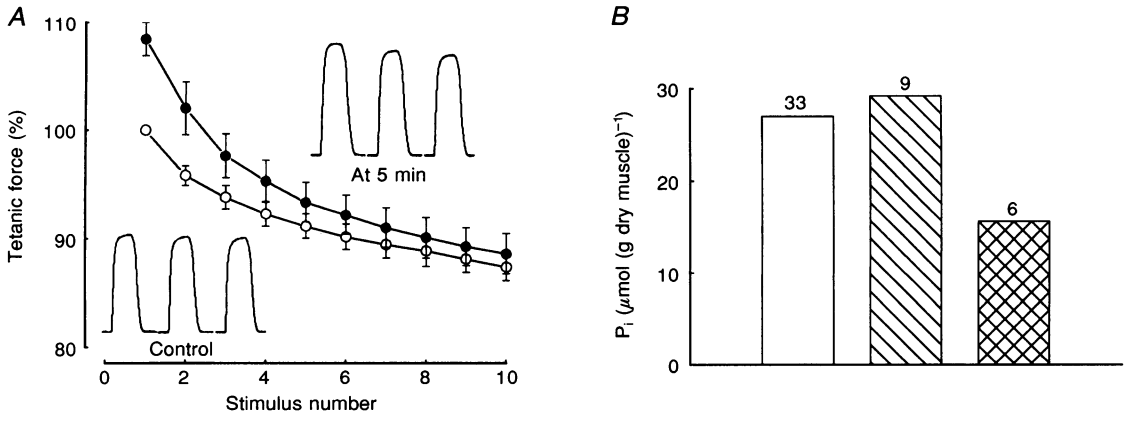

Figure 6. Potentiation is associated with a decrease in P_i

A, force declines more rapidly in a second series of 10 tetani given at the peak of potentiation following the first. Data points represent the mean of 4 fibres. ○, the force decline during a first series of 10 tetani. ●, the decline during a second series which was started at the peak of potentiation. Insets are typical records from 1 fibre showing the decline of force during the early part of the first (Control) and second series (At 5 min). Note the more rapid fall in force during the early part of the second series compared with the first. *B*, inorganic phosphate content is lower in the potentiated than in the rested state; in unstimulated fibres (□), in fibres that were stimulated and left to recover completely (▨) and in fibres that were stimulated and then frozen at the peak of potentiation (▩). Number of fibres in each group is given above the bars.

than control. Thus, these results seem to rule out any role for H^+ in the observed force augmentation

The curvilinear relationship between P_i and force means that increases in P_i depress force production more when the initial P_i concentration is low (e.g. Pate & Cooke, 1989). We postulated that following a series of tetani, P_i had been lowered below the usual resting level. As a consequence, a second series of tetani applied during the period of potentiation, while causing the same absolute rise in P_i as the first series, would result in a greater fall in force over the first two or three tetani. Figure 6*A* shows the decline in tetanic force during two sets of ten tetani applied to the muscle with the second set being applied at 5 min (the time of peak potentiation). Tetanic force clearly declined more rapidly in the early part of the second series than in the first series, supporting the hypothesis that the level of P_i was lower at the start of the second than at the start of the first series.

Measurements of P_i were performed in fibre bundles frozen during the period of augmentation and these were compared with control samples obtained from fibre bundles which had not been stimulated or had been stimulated but allowed to recover completely. Figure 6*B* shows the results of these measurements. In six control samples P_i was $27{\cdot}9 \pm 1{\cdot}8$ μM (g dry muscle)$^{-1}$. However, in the samples frozen at the peak of potentiation, P_i was less than 16 μM (g dry muscle)$^{-1}$, a reduction of more than 40% from that found in control.

DISCUSSION

The present study demonstrates that force production of isolated single muscle fibres can be augmented by a brief period of prior activity. The augmentation of force persists for at least 15 min. We used a steady flow of temperature-controlled solution through the chamber and hence long-lasting changes in intra-fibre temperature can be ruled out as playing a role in this effect. Increased elasticity is also unlikely to be involved as we studied steady-state force in an isolated fibre (see also Fig. 4*B*). Calcium transients during potentiation were reduced, indicating that augmented calcium release plays no role in the observed potentiation. During the period of potentiation, pH_i was reduced. Acidosis is well known to depress tetanic force production in amphibian muscle (e.g. Robertson & Kerrick, 1979; Baker, Brandes & Weiner, 1995) and thus the augmentation in force found in the present study is not due to altered pH_i. The finding that potentiation persists even when fibre swelling has subsided (Fig. 2) suggests that swelling is not the primary factor underlying the observed potentiation.

The potentiation appears to be the result of reducing P_i. Assuming a dry to wet weight ratio of 0·28 (Nagesser, van der Laarse & Elzinga, 1993), P_i in rested fibres was about 7·6 mmol l^{-1}, a value within the range previously reported in amphibian muscle (Godt & Maughan, 1988; Nagesser *et al.* 1993). Following the series of ten tetani, P_i was reduced by about 40% to 4·3 mmol l^{-1}. A comparison with previous data indicates that such a change should result in about an

8–12% increase of tetanic force (Pate & Cooke, 1989; Millar & Homsher, 1990; Stienen *et al.* 1990), which is remarkably similar to the potentiation found in the present study. The mechanism underlying the decrease in inorganic phosphate is unknown but similar undershoots have been seen in rat muscle after incubation in pyruvate (Phillips, Wiseman, Woledge & Kushmerick, 1993) in rat muscle recovering from a 15 min period of 1 Hz stimulation (Kushmerick, Meyer & Brown, 1992), and also in the leg muscles of human subjects recovering from brief periods of moderate exercise (Yoshida & Watari, 1993*b*).

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